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(54) Title: DEVICE AND METHOD FOR DETECTION OF COMPOUNDS WHICH INTERCALATE WITH NUCLEIC ACIDS

(57) Abstract

In a device and method for detection and quantitation of compounds which interact with DNA, the application of evanescent wave technology using a fluorescent intercalating agent as the label offers a built in detection system. The competitive inhibition of an intercalating fluorescent compound by any unknown DNA interactive compound is detectable in real-time by the application of evanescent wave technology utilizing planar or optical fiber architectures.

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attempts to quantitate [illegible] quilibrium dialysis (P acock , R.A. and J.N.H. Skett tt, Trans. Faraday Soc., 52, 261 (1956)) and th rmodynamic

models such as Scatchard plots (Scatchard, G., Ann. N.Y. Acad. Sci., 51, 660 (1949)). Both approaches suggested that one ligand molecule (in this case proflavine) bound for every four or five DNA nucleotides with a high bonding affinity and with little structural perturbation of the DNA.

Since these early studies, many other compounds have been examined by spectroscopy as well as by a variety of other methods including viscosity (Cavalieri, L.F., M. Rosoff, and B.H. Rosenberg, J. Am. Chem. Soc., 78, 5239 (1956)), sedimentation coefficient and NMR measurements (Wilson, W.D. and R.L. Jones, in Intercalation Chemistry, edit. Whittingham, M.S. and A.J. Jacobson, Academic Press, New York, 1982, p. 445-501).

It is well known today that not only do dyes such as the acridines intercalate into DNA (Dinesen, J. and J.P. Jacobsen, F.P. Hansen, E. B. Pedersen, and H. Eggert, J. Med. Chem., 33, 93 (1990), and Zimmerman, S.C., C.R., Lamberson, M. Cory and T.A. Fairley, J. Am. Chem. Soc., 111, 6805 (1989)), but other compounds do so including aminoquinolines (McFadyen, W.D., N. Sotirellis, W.A. Denny, and L.P.G. Wakelin, Biochem. Biphs. Acta., 1048, 50 (1990)); fused aromatics, such as diamino-phenylindoles (Wilson, W.D, F.A. Tanious, H.J. Barton, L. Strekowski, D.W. Boykin, and R.L. Jones, J. Am. Chem. Soc., 111, 5009 (1989)); a large number of polycyclic aromatic hydrocarbons (Harvey R.G., and N.E. Geacintov, Acc. Chem. Res., 21, 66 (1988)); and benzopyrenediol epoxides (Kim, S.K., N.E. Geacintov, H.C. Brenner, and R.G. Harvey, Carcinogenesis, 10, 1333 (1989)), also intercalate with nucleic acids. The table below gives several examples of intercalator classes and representative compounds within each class as noted in Duttagupta, N., U.S. Patent No. 4,777,129, Oct. 11, 1988 and Albarella, J.P., U.S. Patent No. 4,563,417, Jan. 7, 1986, incorporated herein by reference.

DNA INTERCALATOR CLASSES AND REPRESENTATIVE EXAMPLES

Acridine dyes	proflavine, acridine orange, quinacrine, acriflavine
Phenanthrides	ethidium, coralyne, ellipticine, ellipticine cation and derivatives
Phenazines	5-methylphenazine cation
Phenothiazines	chlorpromazine
Quinolines	chloroquine, quinine
Aflatoxin	
Polycyclic hydrocarbons	3,4-benzopyrene, benzopyren and
oxirane derivatives	diol epoxide, benzanthrancene-5, 6-oxide, 1-pyrenyloxirane
Actinomycas	actinomycin D
Anthracyclinones	Beta-rhodomycin A, daunomycin
Thiaxanthenones	miracil D
Anthramycin	
Mitomycin	
Platinum Complexes	
Polyintervalators	echinomycin, quinomycin, triostin, BBM928A, tandem, diacridines, ethidium dimer, and ellipticene dimers, heterodimers and trimers
Norphilin A	
Fluorenes and Fluorenones	fluorenodiamines
Furocoumarins	angelicin, 4, 5-dimethyl-angelicin, psoralen, 8-methoxy-psoralen, 5-aminomethyl, 8-methoxypsoralen, 4, 5, 8-trimethylpsoralen, xanthotoxin, khellin
Benzodipyrones	
Monostral Fast Blue	

Richardson and Schulman, U.S. Patent No. 4,257,774, incorporated herein by reference, disclose an intercalation inhibition assay for compounds that interact with DNA or RNA. This patent requires the use of fluorescent polarization as the method of detection and requires a solution assay.

U.S. Patent No. 4,563,417 to Albarella et al. describes a nucleic acid hybridization assay method for detecting specific polynucleotide sequences. A particular polynucleotide sequence is detected in a test medium containing a single stranded nucleic acid by forming a hybridization product comprising hybridized probe and a nucleic acid intercalator compound bound to double stranded nucleic acid. The invention enables the detection of formed hybrids by inducing an immunogenic modification of double stranded nucleic acid in the region of hybridization. Acridine orange is used as the intercalator compound. Determining the binding of antibody to intercalation complexes can be accomplished in a variety of ways and preferably involves the use of antibody labeled with a detectable chemical group such as an enzymatically active group, a fluorescer, a luminescer, a specifically bindable ligand, or a radioisotope. Albarella et al. use autoradiography or gamma counting as the method of detection.

U.S. Patent No. 5,001,051 to Miller et al. describes a method and apparatus for the <u>in vivo</u> and <u>in vitro</u> detection and measurement of the concentration of nucleic acids which is based on the inherent reaction or perturbation of anti-cancer drugs with DNA. Th m thod is based on the int raction of fluor scent or chromophoric dyes, particularly intercalating dyes, or oth r suitable lab ls which show a dramatic increase in a d tectable signal. Th devic used is a fiber optic sensor, on end of which carries a suitabl support or substrat member, which is capable of binding and retaining the sensing

reagents, and the other end of which is attached or connected to any suitable detection, recording and

analyzing devices. Sensing reagents include compounds which include fluorescent dyes, which may include intercalating dyes. Reactive groups such as a diazo-group facilitate the attachment or covalent bonding of fluorescent dye (ethidium derivative) or DNA to the surface of the glass bead. A change in the fluorescence of the ethidium dimer occurs when the probe is exposed to solutions of various therapeutic drugs. Miller et al. use fiber optic spectrometry as the method of detection.

U.S. Patent No. 4,925,785 to Wang et al. describes a method for carrying out a nucleic acid hybridization test to detect a target nucleic acid sequence (DNA or RNA). The advantages of the method include amplification of detectable signal, ability to employ large numbers of fluorescent labels while avoiding self quenching, simple detection of hybridization without the use of labels, etc. The method of detection of a target nucleic acid of Wang et al. involves detecting changes in turbidity and gelation of the target nucleic acid.

U.S. Patent No. 4,407,942 to Birnbeim describes a fluorescent method for the detection of damage to DNA caused by low doses of radiation. In this method, a selected fluorescent dye is used both to detect double stranded DNA and to monitor its rate of unwinding in alkaline media without the requirement for physical separation of single stranded from double stranded DNA. A kit adapted for carrying out the test method is also described. Fluorescence is measured with a spectrofluorometer.

Blumstein et al., TIBTECH, Vol. 8, June 1990, disclose the use of fiber optic evanescent immunosensors to detect and monitor antigen-antibody reactions. A labelled antigen probe competes with an unknown antigen for a limited number of antigen binding sites on an antibody.

U.S. Patent No. 4,582,809 to Block et al.

includes a radiation source, an optical fiber, means for attaching an immunochemical complex, such as an antibody, to an optical fiber, and a means for detecting radiation. U.S. Patent No. 4,716,121 to Block et al. discloses a method for performing immunoassays using a fluorimeter and total internal reflection technology. The disclosure of Block et al. does not allow for the sensitivity of detection of compounds such as polynuclear aromatic compounds. In addition, immunoassay sensitivity is limited by th binding constant of the antibody and does not allow for the sensitivity of detection achieved with the present invention which is about 100 times greater than immunoassay sensitivity.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for the detection and quantitation of compounds which intercalate with nucleic acids.

It is another object of the present invention to provide a device for detection and quantitation of compounds which intercalate with nucleic acids.

It is another object of the invention to provide a rapid, simple and sensitive method for the detection and quantitation of several varieties of compounds.

It is a further object of the invention to provide for a method for the detection and quantitation of toxic compounds found in air.

Another object of the present invention is to provide a method for the detection and quantitation of compounds found in aqueous environments.

Another object of the invention is to provide a method for the detection of polynuclear aromatic compounds in a sample.

The present invention relates to a device and method for the detection and quantitation of compounds which interact with nucleic acids, such as DNA, using evanescent wave technology. A nucleic acid, including

disclose an apparatus including an optical fib r for fluor scenc immunoassays. Th apparatus of Bl ck t al.

double stranded DNA or RNA, attached to an evanescent wav guide is us d to d tect a known or unknown sample

which intercalates with the nucleic acid by competitive inhibition of a fluorescent label at the solid surface of a waveguide. The evanescent wave technology offers improved sensitivity, speed and simplicity, while the use of a fluorescent intercalating agent as the label offers a built in detection system.

This technique, which utilizes the phenomenon of nucleic intercalation, has the advantage of being a highly sensitive assay for polynuclear aromatic hydrocarbons (PAH's), the assay architecture of which is similar to the architecture of the fluorescent label of the present invention.

The methods and device disclosed herein in accordance with the invention are adaptable for use in many laboratory, industrial, medical, environmental, agricultural, food science and other applications where quantifiable and efficient detection of samples containing known and unknown compounds which intercalate with nucleic acids.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a preferred embodiment for a device for detection and quantitation of compounds which intercalate with DNA. (1) represents a fiber optic waveguide with a nucleic acid bound thereto; (2) represents a sample chamber; (3) represents a light source; (4) represents a focusing means; (5) represents a mirror; (6) represents a photodetector; (7) represents a sample entry port; (9) represents a sample exit port; (---) represents an example of light refraction through the waveguide.

Figur 2 is a schematic diagram of how a sampl competes with an intercalating agent for nucleic acid binding sites. (1) represents a waveguide with a nucleic acid (2) bound thereto; (0) repres nts an intercalating ag nt; (◂) repr s nts a sampl comp ting for nucleic acid binding sites.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

This invention provides a rapid, simple, and sensitive method for the detection and quantitation of several varieties compounds.

The present method may be used to detect samples of known or unknown compounds which intercalate with nucleic acids. Such compounds which are known to intercalate with nucleic acids include but are not limited to acridine dyes, phenanthrides, phenazines, phenothiozines, quinolines, aflatoxin, polycyclic hydrocarbons, oxirane derivatives, actinomyces, anthracyclinones, thiaxanthenones, anthramycin, mitomycin, platinum complexes, polyintervalators, norphilin A, fluorenes, fluorenones, furocoumarins, benzodipyrones, and Monostral fast blue.

Examples of acridine dyes include proflavine, acridine orange, quinacrine, and acriflavine. Examples of phenanthrides include ethidium, coralyne, ellipticine, ellipticine cation, and derivatives thereof. An example of phenothiazine is chlorpromazine. Examples of polycyclic hydrocarbons include 3,4-benzopyrene and benzopyrene. Examples of oxirane derivatives include diol epoxide, benzanthracene-5,6 oxide, and 1-pyrenyloxirane. An example of actinomyces is actinycin D. Examples of anthracyclinone include beta-rhodomycin A and duanomycin. An example of a thiaxanthenone is miracil D. Examples of polyintervalators include echinomycin, quinomycin, triostin, BBM928A, tandem (a tradename for 2-(3,5-dichlorophenyl)-2-(2,22-trichloroethyl)oxirane), diacridine dimers, ethidium dimers, ellipticene dimers, and heterodimers and trimers of elliptic ne. Fluor nes and fluorenones include fluor nodiamines. Furocoumarins include ang licin, 4,5-dimethylangelicin, psoralen, 8-methoxypsoralen, 5-aminom thyl-8-m thoxypsoralen, 4,5,8-trimethylpsoralen, xanthotoxin and khellin. Fluorescent

intercalating agent or label to achiev the objects of th

invention. Preferred fluorescent intercalating agents include ethidium bromide and proflavone.

Bluestein et al. describe well known optical waveguide principles as follows. When a light beam strikes the interface between two transparent media from the optically denser media (n1/n2), total internal reflection (TIR) occurs at angles exceeding the critical angle theta C. The critical angle is defined as theta $C = \sin^{-1}$ (n2/n1). (For further explanation of optical waveguide principles see Block et al., U.S. Patent No. 4,582,809 and U. S. Patent No. 4,716,121 which are herein incorporated by reference.)

In a typical quartz optical waveguide this interface is formed by a high refractive index quartz core and a thin layer or cladding of a lower index glass surrounding the core. While cylindrical fibers are preferred, TIR can occur in a device of any shape, as long as the above optical criteria are met. Other TIR elements may include flat plates and prisms.

The internally reflected light beam is conducted down the waveguide and emerges at the distal end in a defined shape of light (e.g., a cone for a cylindrical fiber) determined by the critical angle of the system. Light emerging from the distal end may be used to probe and monitor chemical reactions of compounds placed in front of it. The transduced signal may include fluorescence, absorption, or light scattering events. In such instances, the optical waveguide is a passive element merely delivering and receiving light in a highly efficient fashion. A number of optical immuno-sensors have been assembled in this configuration and comprise the group known as distal tip sensors.

Inside the optical element, at the interface of incident and reflected light, an electromagnetic field called the vanescent wave penetrates perpendicularly to the fiber axis a small distance (typically less than one

wavel ngth) into the lower index medium. This field
d cays in amplitude xponentially with distance from the

interface of the two optically diff rent m dia. The depth of penetration is a measure of the distance that defines where the amplitude of the evanescent wave has decayed 1/e of its magnitude at the interface - this is approximately 36% of the initial intensity. The equation determining the depth of penetration involves the wavelength, the angle of incident light and the refractive index ratios of the two optically different media. When water or serum replaces the glass cladding and acts as the low index optical media, at an incident wavelength of 500 nm., the depth of penetration is about 100nm.

The implications of this confined wave for conversion of a passive light conductor to a sensing transducer of a compound which intercalates with a nucleic acid according to the present invention are profound. First, due to the constricted energy field, only optical compounds in the immediate vicinity of the surface of the fiber will be detected - unbound molecules in distant regions of the solution will not be illuminated. Thus, the need for extensive washing prior to measurements being taken is eliminated. Second, since a nucleic acid is immobilized directly to the lateral surfaces of the quartz fiber, the waveguide itself becomes a mass concentrating device capable of simultaneously binding, amplifying and detecting molecules bound to the nucleic acid. Reactions are initiated by immersing the fiber into a sample and an intercalating agent. Molecules in bulk solution beyond the evanescent zone are not detected.

If a fluorescent derivative such as fluorescein is coupl d to a nucleic acid, longer wavelength fluor s- cent emissions of fluors at the interface will tunnel their way back into the fib r with an efficiency of about 2%. Thus, when optimizing the amount of energy put into the evanescent zone, it is important that as much light as possible approaches the critical angle. S nsitivity of detection is either gained or lost very significantly as

the angle of input or output approach s or recedes fr m the critical angl . Thus it is d sirable to hold the

sensor and the sample chamber without perturbation or loss of the high angle of the excitation emission light.

Sensitivity of detection is limited by the uniformity of the waveguide, minimization of light scatter and the amount of fluorescent light due to unbound fluorophors that makes its way into low angle emission.

Fiber optic sensors offer the greatest sensitivity for sample detection. Fiber optic sensors avoid spreading and loss of light in the transverse direction. Cylindrical symmetry of the fiber optic waveguide allows the entire surface area to be used for sensing and collection of light. A large number of internal reflections in the fiber optic waveguide allows signal enhancement as every intercession of incident light with the surface generates a signal-creating evanescent wave, and fluorescence that tunnels back into the fiber is concentrated in a bright spot on a small detector face, resulting in less electrical noise in the signal amplification process.

Further reductions in unwanted background, such as unbound molecules in distant regions of a sample solution, may be achieved if the intercalating agent on the light collecting surface of the fiber used is the same as that into which excitation light is launched. In this case only residual excitation light from end-surface reflections needs to be filtered. When the detector is placed at the distal end, where most of the light emerges, extensive spectral filtering is required in order to detect evanescent fluorescent emission. 1 mm diameter quartz rods may be used which are 50 mm in length.

The method for the detection and quantitation of compound which intercalate with a nucleic acid includes placing a fluorescent intercalating agent and a sample in close proximity to a waveguide coated with a nucleic acid. The sample, nucleic acid and fluorescent intercalating agent are allowed to react, such that the sample competes with the fluorescent intercalating agent for nucleic acid

binding sites. An xcitation light is pass d through th waveguide with a nucleic acid bonded th reto to excite the

fluorescent intercalating agent c ming within th van s-cent wave with the excitation light. Radiated light is detected from the fluorescent intercalating agent at the initiating end with a photodetector. The radiated light is compared to a similar waveguide and fluorescent intercalating agent value previously run in the absence of a sample.

The agents (sample, nucleic acid and fluorescent intercalating agent) may be added separately or together or in any desired order. The time for obtaining a signal may range from up to 30 seconds to 30 minutes or more. If the signal in the presence of an unknown sample is significantly less than the control signal, then one can assume that an intercalator molecule was present in the unknown sample.

Calibration is carried out by measuring a series of known concentration standards in the same fluid matrix as the unknown. The signal response parameter of the unknown sample is then quantified by extrapolation from a linearized standard curve.

Increased sensitivity may be obtained by "pulsing" the sample with fresh buffer to remove unreacted fluorescent intercalating agent from the vicinity of the fiber. The buffer should be of a pH and ion concentration which will not cause release of bound fluorescent molecules or sample from the nucleic acid and should allow for maximum fluorescence of the fluorescent molecule. The sample fluid may be buffered in the ranga of pH 6-9, preferably pH 7-8. Various buffers such as borate, tris, carbonate and the like may be employed.

In a pref rred embodiment, an unknown aqueous sample is add d to a nucleic acid containing fiber attached to the waveguid devic , surrounded by a capillary like tube which holds th liquid. The sample may be as small as 10 to 100 microlit rs. The tube containing the sample may also contain dried fluor scent intercalat-

ing agent, in micromolar amounts, which b comes dissolved in the sample. The molar concentration of th fluor scent

intercalating agent is preferably 10^{-3} to 10^{-6}. The reagents are allowed to react until enough signal is observed to obtain a difference between a similar fiber and fluorescent intercalating agent value previously run the absence of any unknown.

The fluorescent intercalating agent includes compounds selected from the above listing of compounds which are known to intercalate with nucleic acids. The fluorescent intercalating agent is selected so that it possesses a different emission spectra than the compound to be detected in the sample. A skilled artisan will be able to select the appropriate fluorescent intercalating agent.

The present invention in a preferred embodiment includes a method for the detection and quantitation of compounds found in aqueous environments.

The preferred sample size for detecting compounds present in aqueous environments is 10 to 100 microliters. The preferred reaction time between the intercalating agent, nucleic acid and sample is 30 sec. to 30 min.

An intercalation assay utilizing a solution format and fluorescence polarization methodology showed an average sensitivity of 5-10 picomoles/ml in the assay. This represents a concentration of 10^{-15} moles of compound detected/ml of solution tested. The results of the present invention show a 100-fold improvement over thes data for DNA detection experiments, also using ethidium bromide as the fluorescent intercalating agent.

Most assays proposed today for th rapid detection of organic compounds at low concentration involv use of monoclonal antibodies specific for the antigen (organic compound) of int rest. The use of antibody assay t chnology for th detection and quantitation of pesticides and polynuclear aromatic hydrocarbons (PAH's) has s v ral important drawbacks which should be considered. First

antibodies ar by their natur highly specific. This is useful if one wants to detect a specific compound, but not

useful if one wants to detect a class of compounds. In the case of airborne carcinogens such as pesticides, th known methods require the collection and analysis of pesticides generally over 24 hour air sampling times plus analysis time.

The current method recommended by the EPA requires 24 hour sample collections utilizing air sampling devices which sample approximately 5-6m^3 of air. Current analysis utilizes gas chromatograph/mass spectroscopy (GC/MS) or gas chromatograph/electron dapture detection (GC/ECD) for quantification. Current methods of analysis of semi-volatile polynuclear aromatic hydrocarbon compounds (PAH's) are even more difficult, requiring filter collection of 15-20 L/min of air for 12-24 hours (150 m^3) to provide sufficient PAH's for detection using GC/MS (electron or chemical ionization).

Minimal detectable levels of pesticides by known methods require from 1-100 ng/m^3 residues in air collected, while with PAH's the level is in the range of 0.1 ng/m^3 of collected air. The total required amount of sample for detection of pesticides is somewhere between 5-500 ng of pesticide residues, while for PAH's the absolute quantities required are in the 15-150 ng levels.

The present invention has the advantage of being a highly sensitive assay for airborne pesticides and PAH's.

Thus, when applied to the detection and quantitation of a compound found in air, the method of the invention may include placing an intercalating agent and a sampl of air which has been dissolved in a liquid solution adjacent to a waveguide coat d with a nucleic acid. The sample, nucleic acid and intercalating ag nt are allowed to r act, such that the sample compet s with the intercalating ag nt for nucleic acid binding site. An excitation light is passed through the waveguide to excit the intercalating agent coming within an evanescent wave

of the xcitati n light. Radiat d light from th interca-
lating agent is d t ct d at th initiating nd of th

waveguide, and th radiated light is compared to a similar waveguide and intercalating agent value previously run in the absence of a sample.

The present method allows detection and quantitation of toxin levels of 10^{-17} moles to 10^{-15} moles with little difficulty. This being the case, the quantity of air required would be less than 1/1000th of the presently required 5-50 m^3 of air now collected. At the rate of 4-40 liters/min this means that collection times could b cut to approximately 2-20 minutes total time. Since the air collection time is so short, it should be possible to collect air directly into the assay solution, thus avoiding the filter collection and subsequent organic extraction procedures required for GC/MS analysis.

A sample of air may be collected which comprises between 4-40 liters/min of air, with a concentration of 0.1-500 ng/m^3 for analytes of molecular weights in the 100-500 Dalton range. The concentrations of interest would be approximately 0.01 to 1.0 nanomole/m^3 of collected air.

Using double stranded DNA and ethidium bromine as the intercalating dye, detection of compounds which intercalate with DNA is possible at the level of 10 attomoles. Similar levels of the intercalating compounds could be detected using similar instrumentation and methodologies.

An assay based on this principal is rapid, simple, and highly sensitive.

In the case of pesticides and PAH's, one may be mainly interest d in determining th classes of compounds in a simpl , rapid on site ass y proc dure. Th second consid ration is sensitivity. Immunoassay sensitivity is limited by th binding constant of th antibody as described in the general mass action equation. Sinc antibody binding constants average in th 10^7 to 10^9 L/mole range, the sensitivity of these known assays is

generally limited to the 10^{-8} to 10^{-10} mol s/L range. For most compounds this represents the low ng/ml concentration

range. In the case of PAH's and pesticides, this sensitivity range is sufficient for analysis of 24 hour collection, but not sufficient for short on the spot analysis where sensitivity in excess of 100 fold is required. Antibodies are capable of detecting specific compounds at concentrations equivalent to concentrations detected by GC/MS methods. The intercalation of a compound with nucleic acids as in the present method using an evanescent waveguide device allows for greater sensitivity of samples containing lower concentrations of compounds.

Therefore, the present invention also includes a method for the detection of polynuclear aromatic compounds in a sample, includes placing an intercalating agent and a polynuclear aromatic compound sample in close proximity to a waveguide coated with a nucleic acid. The sample, nucleic acid and intercalating agent are allowed to react, such that the sample competes with the intercalating agent for nucleic acid binding sites. An excitation light is passed through said waveguide to excite the intercalating agent coming within an evanescent wave of said excitation light. Radiated light from the intercalating agent is detected at the initiating end of the waveguide, and the radiated light is compared to a similar waveguide and intercalating agent value previously run in the absence of a sample.

A device for detection and quantitation of compounds which intercalate with nucleic acids of the present invention may include a chamber for a sample to be tested (2); a waveguide with a nucl ic acid bonded thereto (1), wherein the waveguide is proximally locat d to the sample in the chamber. A light source (3) at a first end of th waveguide is capable of shining monochromatic light at a wavelength of excitation of an intercalating agent bound to the nucleic acid, and of passing an xcitation light through the waveguide via evanescence. A photodetector

(6) at the first end of the waveguide d t cts radiat d

light emitted from said intercalating agent excited by the light source.

In a preferred embodiment (FIG.1) the device may also include a focuser such as a lens or the like for focusing light from the light source through the waveguide. The focuser may be separate from the waveguide, as in a lens (4), or may be a portion of the waveguide.

In an additional preferred embodiment the device may include a mirror (5) angularly disposed between the focuser and the photodetector. The mirror may preferably be a dichroic mirror, and may act as a low pass interference filter with a cut off frequency chosen to be between the frequencies of maximum absorption and maximum fluorescence emission of the fluorescent intercalating agent of interest.

The waveguide may preferably be composed of glass, silica, quartz, ceramic, plastic, polyolefin, nylon or any polymer having a suitable refractive index. The waveguide may preferably be an optical fiber, slide or planar plate. In a preferred embodiment, a fiber optic waveguide has a diameter in the range of 200 microns to 1 mm and a length of from about 25 mm to 50 mm.

The light source may preferably be a tungsten halogen lamp, a mercury lamp, a flash lamp, or a laser. The light source is chosen to maximize the fluorescence of the fluorescent intercalating agent. Silicon diodes may preferably be used as the photodetector.

In addition a ratio amplifier may be connected to the output of the photodetector and further connected to an input for display. Display may be achieved by any of a number of devices which provides a visual signal representative of an electrical input and may be, for example, a digital display, a strip chart recorder or a meter.

A waveguide preferably made of glass, silica

quartz, polyol fin, nylon or plastic may be coat d with a nucleic acid via silanization, covalent coupling or direct

absorption. The waveguide is chosen to have a refractive index greater than that of the sample. An aqueous sample typically has an index of refraction near 1.33 and a serum sample has an index of refraction near 1.35. If a plastic waveguide is used, the plastic material must be of low florescence, and optically clear.

A method for preparing the surface of the waveguide, of attaching a silyl compound thereto, and of covalently bonding a nucleic acid to the glass via silyl coupling is described in Weetall, U.S. Patent No. 3,652,761, which is incorporated herein by reference. Other known techniques are also available for attaching a nucleic acid to a solid support.

The nucleic acid may take the form of double stranded DNA, double stranded RNA, a DNA/RNA complex or hybrid molecule, or natural or synthetic analogue, fragment or derivative thereof. The amount of nucleic acid required to coat the waveguide is about 1 microgram or less. The nucleic acid should be in a form which is easily obtainable. Any nucleic acid of known sequence is appropriate. The use of a linearized plasmid is preferred; however oligonucleotides of known sequence may also be used. Suitable nucleic acids may be obtained from Sigma Chemical Co., St. Louis, Missouri.

For example, double stranded DNA can be laid down in such a fashion that the majority of the molecules lay along the surface of the waveguide. The waveguide is washed with buffer to remove excess DNA and dried until needed. In cases where covalent coupling is employed, the wash buffer should be of high ionic strength (0.5M-2.0M) in order to remove adsorbed DNA. In the case of an adsorbed DNA coupled system, the wash buffer should be of low ionic strength, preferably 0.01-0.1M. Buffers preferably include phosphate, carbonate and borate, for example.

An optical fiber is placed in a device which is capable of shining monochromatic light at the wavelength

of xcitation of the fluoresc nt intercalating agent. A sampl is plac d in clos proximity to the fib r, as shown

in Figure 1. The xcitation light passes through th fiber via evanescence. Any fluorescent intercalating agent coming within the evanescent wave is excited and radiates light back through the fiber and this light is detected at the initiating end of the fiber by a photodetector. Since the photodetector and light source are constantly in operation, the binding of the fluorescent intercalating agent can be monitored as it occurs. Th addition of a known or unknown sample to this system which is capable of intercalating with the nucleic acid will cause competition for the available binding sites. Therefore the more sample present, the less fluorescent intercalating agent will bind. The less known or unknown sample present, the more the fluorescent intercalating agent will bind to the waveguide coated with a nucleic acid.

EXAMPLE 1

1 micromole of ethidium bromide in a 10 microliter sample of rainwater is placed in contact with a silica fiber optic waveguide of 1 mm in diameter and 50 mm in length (obtained from Corning, Incorporated, Corning, N.Y. or ORD, Corp. Nashua, NH). The waveguide is coated with double stranded DNA (obtained from Sigma Chemical Co.).

The sample, double stranded DNA and ethidium bromide are reacted for 15 minutes.

Light from a tungsten halogen lamp which emits light of a wavelength of 500 nm is passed through the fiber optic waveguide.

A lens is employed to image the light source on th end fac of the fib r optic waveguide, and to coll ct all radiation exiting th end fac of th fiber optic waveguide and direct th radiat d light at the photodet ctor.

Between th light source and the lens is plac d a dichroic mirror which acts as a light filter.

A silicon diod phot d tect r detects radiat d light du to th binding of ethidium bromid . The value

of the radiated light is compared to a standard value to reveal the amount of the sample. The sample is found to contain 2.0 ng/ml of sample when comparing the radiated light to a similar waveguide and ethidium bromide value previously run in the absence of a sample.

EXAMPLE 2

1.5 micromole of acriflavine (obtained from Aldrich Chem. Co., Milwaukee, Wisc.) in a 15 microliter sample of groundwater is placed adjacent to a silica fiber optic waveguide of 1 mm in diameter and 50 mm in length (obtained from ORD Corp., Nashua, NH). The waveguide is coated with double stranded DNA.

The sample, double stranded DNA and acriflavine are reacted for 5 minutes.

Light from a tungsten halogen lamp which emits light of a wavelength of 500 nm is passed through the fiber optic waveguide.

A lens is employed to image the light source on the end face of the fiber optic waveguide, and to collect all radiation exiting the end face of the fiber optic waveguide and direct the radiated light at the photo detector.

Between the light source and the lens is placed a dichroic mirror which acts as a light filter.

A silicon diode photodetector detects radiated light due to the binding of acriflavine. The value of th radiated light is compared to a standard value to reveal the amount of the sample. The sample is found to contain 15 ng/ml of sample comparing the radiated light to a similar waveguide and acriflavine value pr viously run in th absenc of a sample.

EXAMPLE 3

2 micromol of profl von in a 10 micr liter sample of blood is placed adjac nt to a gl ss fiber optic wav guid of 1 mm in diamet r and 50 mm in l ngth

double stranded RNA.

The sample, doubl strand d RNA and proflavon are reacted for 8 minutes.

Light from a mercury lamp which emits light of a wavelength of 500 nm is passed through the fiber optic waveguide.

A lens is employed to image the light source on the end face of the fiber optic waveguide, and to coll ct all radiation exiting the end face of the fiber optic waveguide and direct the radiated light at the photo detector.

Between the light source and the lens is placed a dichroic mirror which acts as a light filter.

A silicon diode photodetector detects radiat d light due to the binding of proflavone. The value of th radiated light is compared to a standard value to rev al the amount of sample. The sample is found to contain 10 ng/ml of sample when comparing the radiated light to a similar waveguide and proflavone value previously run in the absence of a sample.

EXAMPLE 4

A 10 liter/min sample of air is collected for a period of 5 minutes through a bubble trap containing 10 ml of 0.01 M phosphate buffer and placed into a sealed chamber.

A sample containing 100 µl of this solution is placed is placed adjacent to a quartz fiber optic wav - guide of 1 mm in diameter and 50 mm in length (obtained from ORD Corp.). The waveguide is coated with double stranded RNA (obtained from Sigma Chemical Co).

2 micromoles of 10^{-5} M acridin orang (obtained from Aldrich Chemical Co.) as th intercalating agent is add d to the waveguide and sample. Th dissolved air sampl , acridine orange and RNA are reacted for 30 minutes.

Light from a tungsten halogen lamp which emits light of a wavelength of 500 nm is passed through the

fib r ptic wav guide.

A silicon diode photodetector detects radiated light due to the binding of acridine orange. The value of the radiated light is compared to a standard value to reveal the amount of the sample. The sample is found to contain 0.1 ng/ml of added sample when comparing the radiated light to a similar waveguide and acridine orange value previously run in the absence of a sample.

EXAMPLE 5

A 20 liter/min sample of air is collected through a 10 ml bubble trap as previously described for a period of 10 minutes and placed into a sealed chamber. The air is dissolved in 40 microliters of distilled water for 10 minutes.

A 100 µl sample in the buffer is placed in contact with a glass planar waveguide (Glass Microscope cover slip) of 1 mm in thickness and 50 mm in length (obtained from Fisher Scientific). The waveguide is coated with a DNA/RNA hybrid.

1 micromole of ethidium bromide as the intercalating agent is added to the waveguide and sample. Th dissolved air sample, ethidium bromide and RNA are reacted for 15 minutes.

Light from a tungsten halogen lamp which emits light of a wavelength of 500 nm is passed through the fiber optic waveguide.

A silicon diode photodetector detects radiated light due to the binding of ethidium bromide. The value of the radiated light is compared to a standard value to reveal the amount of the unknown sample. The sample is found t contain 0.01 ng/ml f unknown sampl when comparing the radiated light to a similar waveguide and thidium bromide value pr viously run in the absenc of a sampl .

As will be appar nt to those skilled in th art in light of the foregoing disclosure, many alterations, modifications and substitutions ar possible in the

spirit r sc pe ther of.

WHAT IS CLAIMED IS:

1. A device for detection and quantitation of compounds which intercalate with nucleic acids, comprising:

a) a chamber for a sample to be tested,

b) a waveguide with a nucleic acid bonded thereto, wherein said waveguide is proximally located to said chamber,

c) a light source at a first end of said waveguide, said light source being capable of shining monochromatic light at a wavelength of excitation of a fluorescent intercalating agent bound to said nucleic acid, and of passing an excitation light through said waveguide via evanescence,

d) a photodetector at said first end of said waveguide, for detecting radiated light emitted from said intercalating agent excited by said light source.

2. The device of claim 1, further comprising a focusing means for focusing light from said light source on said waveguide.

3. The device of claim 1, further comprising a mirror angularly disposed between said focusing means and said photodetector.

4. The device of claim 1, wherein said nucleic acid is bonded to said waveguide by silanization.

5. The device of claim 1, wherein said nucleic acid is bonded to said waveguide by covalent coupling.

6. The device of claim 1, wherein said nucleic acid is bonded to said waveguide by direct absorption.

7. The device of claim 1, wherein said chamber is a capillary tube.

8. The device of claim 2, wherein said focusing means is a portion of said waveguide.

9. A method for the detection and quantitation of compounds which intercalate with nucleic acids compris-

a) placing a fluorescent intercalating agent and a sample in close proximity to a waveguide coated with a nucleic acid,

b) allowing the sample, nucleic acid and intercalating agent to react, such that said sampl competes with said intercalating agent for intercalating with binding sites of said nucleic acid,

c) passing an excitation light through said waveguide to excite said intercalating agent coming within an evanescent wave of said excitation light,

d) detecting radiated light from said intercalating agent at the initiating end of said waveguide, and

e) comparing said radiated light to a similar waveguide and intercalating agent value previously run in the absence of a sample.

10. The method of claim 9, wherein said nucleic acid is double stranded DNA.

11. The method of claim 9, wherein said nucleic acid is RNA.

12. The method of claim 9, wherein said intercalating agent is selected from the group consisting of acridine dyes, phenanthrides, phenazines, phenothiozines, quinolines, aflatoxin, polycyclic hydrocarbons, oxirane derivatives, actinomyces, anthracyclinones, thiaxanthenones, anthramycin, mitomycin, platinum complexes, polyintervalators, norphilin A, fluorenes, fluorenones, furocoumarins, benzodipyrones, monostral fast blue.

13. The method of claim 9, further comprising the step of pulsing the sample with a fresh buffer to remov unreact d fluor sc nt int rcal ting ag nt from the vicinity of said waveguide to incr ase the sensitivity of d tection of said sampl .

14. Th m thod of claim 9, wh r in said sample is d t cted at a minimum lev l of 10 attomoles.

15. The m thod of claim 9, wh r in said wave-

16. The m thod of claim 9, wher in said wav - guide is omposed of plastic.

17. The method of claim 9, wherein said wav - guide is an optical fiber.

18. The method of claim 9, wherein said wave-guide is a plate.

19. The method of claim 9, wherein said compounds are polynuclear aromatic compounds.

20. The method of claim 9, wherein said sample of air dissolved in a liquid comprises polynuclear aromatic compounds.

21. The method of claim 9, wherein said sampl is 10 to 100 ml in size.

22. The method of claim 9, wherein said sample, nucleic acid and intercalating agent is allowed to react for 30 sec. to 30 min.

23. A method for the detection and quantitation of compounds found in air, comprising the steps of:

a) placing a fluorescent intercalating ag nt and a sample of air which has been dissolved in a liquid solution to dissolve said compound to be tested adjacent a waveguide coated with a nucleic acid,

b) allowing said sample of air, nucleic acid and intercalating agent to react, such that said sample competes with said intercalating agent for intercalating with binding sites of said nucleic acid,

c) passing an excitation light through said waveguide to excite said intercalating agent coming within an evanescent wave of said excitation light,

d) detecting radiated light from said intercalating agent at the initiating end of said waveguide, and

) comparing said radiated light to a similar waveguide and int rcalating agent value previously run in the absenc of a sample.

24. The m thod of claim 23, wherein said compounds ar toxic compounds.

PCT/US92/09916

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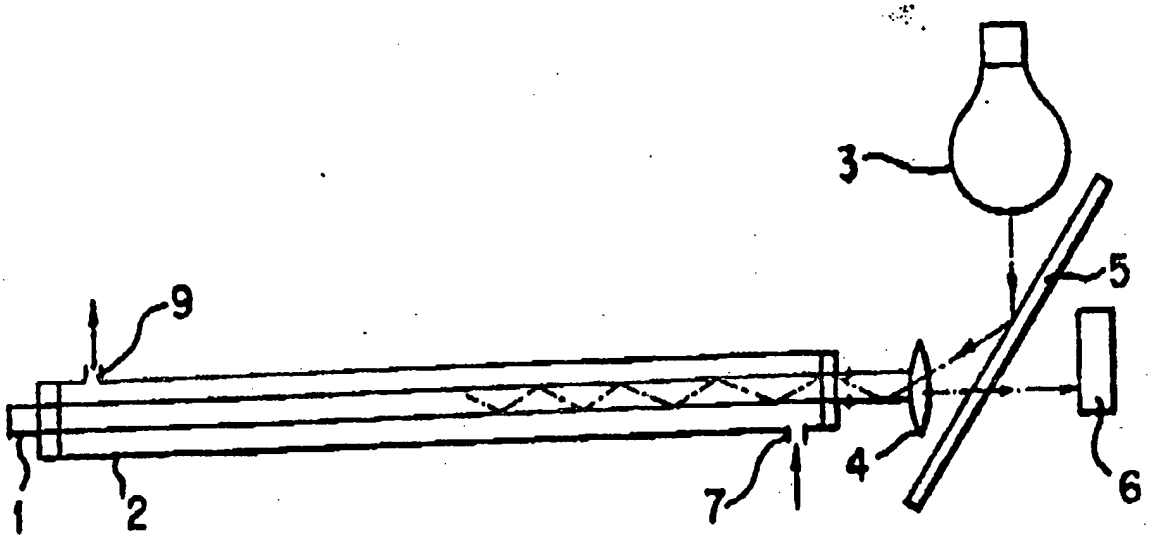

FIG. 1

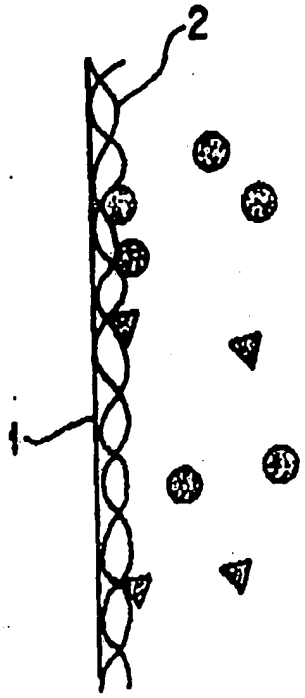

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09916

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; C12M 1/34
US CL :435/6, 291

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 291

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG: waveguide, nucleic acid, intercalate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,257,774 (RICHARDSON ET AL.) 24 March 1981, column 2, line 57 - column 3, line 54.	1-24
Y	US, A, 5,001,051 (MILLER ET AL.) 19 March 1991, Abstract.	1-24
Y	US, A, 4,777,129 (DATTAGUPTA ET AL.) 11 October 1988, column 4, lines 9-33.	1-24
Y	US, A, 4,563,417 (ALBARELLA ET AL.) 07 January 1986, Summary Of The Invention.	1-24
Y	EP, A, 0,245,206 (SUTHERLAND ET AL.) 30 April 1987, Abstract.	1-24
Y	Tibtech, Vol.8, issued June 1990, BLUESTEIN ET AL., "Fiber Optic Evanescent Wave Immunosensors For Medical diagnostics" page 1-8, entire document.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

03 MARCH 1993

11 MAR 1993

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TOTAL P.30